

## TAXONOMIC STATUS OF *DIDYMIUM LAXIFOLIUM* AND *D. RUBEOPUS*, INCL. A NEW VARIETY OF *D. RUBEOPUS* (MYXOMYCETES).

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**ABSTRACT** — *Didymium rubeopus* is described, together with the new variety *D. rubeopus* var. *albocapillitium*. It is compared with the similar *D. laxifilum*, for which a lectotype is proposed. LM and SEM microphotographs illustrate the more representative characters.

**KEY WORDS** — *Didymium laxifilum*, *D. rubeopus*, *D. rubeopus* var. *albocapillitium*, Myxomycetes, taxonomy.

**RESUMEN** — Se describe *Didymium rubeopus* y la nueva variedad *D. rubeopus* var. *albocapillitium*. Se compara con la especie próxima *D. laxifilum* de la que se propone un lectotipo. Se aportan microfotografías de M.O. y MEB de sus características morfológicas más representativas.

**PALABRA CLAVAS** — *Didymium laxifilum*, *D. rubeopus*, *D. rubeopus* var. *albocapillitium*, Myxomycetes, taxonomy

**RÉSUMÉ** — On décrit *Didymium rubeopus* et la nouvelle variété *D. rubeopus* var. *albocapillitium*. On compare avec une espèce proche *Didymium laxifilum* de laquelle on propose un lectotypus. On propose des microphotographies au MO et au SEM de leurs caractéristiques morphologiques les plus représentatives.

**MOTS-CLEFS** — *Didymium laxifilum*, *D. rubeopus*, *D. rubeopus* var. *albocapillitium*, Myxomycetes, taxonomie.

### INTRODUCTION

*Didymium laxifilum* was described by Gulielma Lister and Joseph Ross (Lister, 1945) from abundant material collected in Epping Forest near the Warren, Loughton (Sussex, England) on leaves of beech and bramble. The first collection was made by Ross in February, 1935, who found it to be generally plentiful in Epping Forest and adjacent

areas in the autumn of 1943. G. Lister and J. Ross also examined further gatherings collected by Mr. W.D. Graddon near Woodford Wells (in the Waltham Forest district of North London and ca. 3 km south of the Epping Forest locality). However, despite the abundance of collected material, no type was indicated with the original diagnosis.

Kowalski (1973), during a visit to the Royal Botanic Gardens, Kew (England), examined a collection labelled as: *Didymium laxifilum* G. Lister & Ross, Ross 3501, Loughton, Epping Forest (Sussex, England). According to Kowalski, this is apparently the type collection.

In 1993, Mr. J.R. Garcia sent us some collections of an apparent *Didymium laxifilum*, found on leaves of *Quercus ilex*, in the Spanish provinces of Badajoz and Córdoba. With the object of comparing these samples with authentic *D. laxifilum* we applied to the Kew Herbarium for loan of the material determined by G. Lister, and, in particular, the collection 3501 studied for Kowalski, which is proposed for us as the lectotype.

After comparing the type material of *Didymium laxifilum* with the Spanish collections, we decided that both had different characters, which stimulated us to propose it as a new species for science. The new taxon (*D. rubeopus*) was validly published in the Abstracts of 2nd. Intern. Syst. Ecol. Myxomycetes (Moreno & al., 1996). Subsequently, we were able to study additional material of *D. rubeopus* from various localities in Spain, France and Mexico, which we found to be differentiated by their capillitium, which has lead us to propose a new variety of the latter taxon. Included are the original Latin diagnoses of *Didymium laxifilum* and *D. rubeopus*, which were published in journals which may be difficult to obtain.

## MATERIAL AND METHODS

Light microscopy (LM) was made with a Nikon microscope equipped with an automatic photographic system. Samples for these studies were mounted in Hoyer's medium. The SEM micrographs have been made with a Zeiss DSM-950 microscope. Spores samples were rehydrated with concentrated ammonium hydroxyde (28-30%) for 30 min, then dehydrated in aqueous ethanol (70%) for 1-1.5 hrs., afterwards immersed in pure acetone for at least 2 hours. Finally, the spores were fixed in formaldehyde dimethylacetal followed by critical point drying and sputter-coating in gold-palladium.

The material of *Didymium laxifila* and *D. rubeopus*, is preserved in the herbarium of the Universidad de Alcalá (AH). The lectotype of *D. laxifilum* is in K.

## TAXONOMIC TREATMENT

*Didymium laxifilum* G. Lister & Ross in G. Lister, *Essex Naturalist* 27: 164. 1945. Figs. 1-23.

= *Didymium aurantipes* Brooks & Kowalski, *Mycologia* 58: 169-170.

**Collections examined.** England: Loughton (Epping Forest), on dead leaves, leg. G. Lister & H.J. Howard, 19.I.1944, n° 3501 (this collection is designated as the LECTO-TYPE and is deposited in K). Loughton, the Warren 16702. Spain: Badajoz, Azuaga, on leaves of *Quercus ilex*, leg. J.R. Garcia, 22.I.1992, AH 16429. Badajoz, Peraleda del Zaucejo, on leaves of *Quercus suber*, leg. J.R. Garcia, 30.I.1994, AH 16725. Córdoba,

Fuenteovejuna, on leaves of *Quercus ilex*, leg. J.R. García, 15.I.1994, AH 14966. Málaga, road Cortes de la Frontera-Alcalá de los Gazules, on leaves of *Quercus suber*, leg. A. Ortega, M.T. Vizoso, E. Gallego, F. Esteve & C. Illana, 12.XII.1990, AH 13382, 13383 and 13385.

**Latin diagnosis.** *Peridiis sparsis vel subconfluentibus, profunde umbilicatis; stipitibus tenuibus, gilvis vel flavo-gilvis; columella hemispherica, floccis capillitii robustis, laxis, subsimplicibus vel in reticulo junctis; sporis fuscis, delicate verruculosis, 9-11 µm diam., area dehiscentiae pallida laeve.*

Sporocarps 0.5-1 mm diam., in small groups, subglobose, sometimes joined together, forming short plasmodiocarps, strongly umbilicate at the base, stipitate or sessile, whitish in colour. Hypothallus discoid, reddish. Stipe up to 0.5 mm tall, reddish, fibrous and without calcium carbonate crystals. Peridium simple, membranous, iridescent, with abundant deposits of white or yellowish calcareous crystals, irregularly dehiscing. columella white, hemispherical.

Capillitium consisting of thick filaments (5-8 µm diam.) which radiating from the columella, branching and anastomosing into a three-dimensional net, violaceous brown to dark brown, hyaline at the ends, with the surface smooth by SEM. Spores 10-13 µm diam., black in mass, very dark purple-brown under the microscope with a pale zone, spherical, verrucose. By SEM, the spores show an ornamentation formed of warts which are joined to form short crests completely covering the spore surface.

*Didymium rubeopus* var. *rubeopus* G. Moreno, Castillo & Illana, in Moreno, Castillo, Illana & Lizárraga, *Abstr. 2nd. Intern. Congr. Syst. Ecol. Myxomycetes*: 57. 1996. Figs. 24-33.

**Collections examined.** Spain: Córdoba, Fuenteovejuna, on leaves of *Quercus ilex*, leg. J.R. García, 11.XII.1993, AH 16444 and 16458 (holotype). Badajoz, Peralda del Zaucejo, on leaves of *Quercus suber*, leg. J.R. García, 13.XII.1993, AH 15221 and 18505.

**Latin diagnosis.** *Sporangia 0.4-1 mm diam., globulus, umbilicatus, albus vel pallide griseus; stipes parvus, usque ad 1 mm alt., rufus vel rufo-aurantiacus, crystalli absens. Peridium fuscus iridescens, crystalli calcari abundantibus exornatus. Columella alba, globosa. Capillitium fuscus vel obscurus, verus peridium hyalinus ad columella radiantibus, filiis tenuis, plus minusve parallelis, forse ramificatis, regularis, flexuosis, hic inde incrassatus, noduli reteque adsens. Sporis 9-11 µm diam., globosis, atroviolaceis, verrucosis. Plasmodium ignotus.*

Sporocarps 0.4-1 mm in diam., stipitate to sometimes sessile, scattered, globose or subglobose, white or light grey in colour, umbilicate. Short stipe, approximately of similar height as the sporotheca, reddish or reddish-orange, translucent, longitudinal wrinkles and without calcium carbonate crystals. Peridium simple, iridescent, covered with abundant whitish crystals, irregularly dehiscing. Hypothallus concolorous with the stipe, forming an extended base. Columella white, globose or hemispherical.

Capillitium formed by delicate, flexuous filaments (2-4 µm diam.) of equal width for their total length, more or less parallel, with some branching, which radiate from the columella, dark grey, hyaline towards the exterior, and with some globose swelling, without forming nodules or a well-defined net, with the surface rugose by SEM. Spores

9-11  $\mu\text{m}$  diam., black in mass, dark brown-violaceous under the microscope, globose, spiny. By SEM, the spore ornamentation is formed by 0.5  $\mu\text{m}$  tall pila, sometimes united, which homogeneously cover the whole surface. Plasmodium not observed.

*Didymium rubeopus* var. *albocapillitium* G. Moreno, Castillo, Illana & Lizárraga, var. nov. Figs. 34-40.

**Collections examined.** Spain: Badajoz, Azuaga, on leaves of *Quercus ilex*, leg. J.R. García, 1.III.1994, AH 16736. Almería, Turrillas, on leaves of *Quercus ilex*, leg. V. González, A. Altés, C. Illana, 2.XII.1993, AH 16373, 16375, 16376, 16379 (holotype), 16383 and 16387. Almería, Turrillas, on leaves of *Quercus ilex*, leg. G. Moreno, A. Altés & C. Illana, 25.II.1994, AH 16649.

France: St. Martin de la Brasque, 12.XI.1994, herb. Meyer 15042 (duplo in AH 18391). Roquemaure-Aire autoroute, 22.IV.1996, herb. Meyer 16307 (duplo in AH 18392).

Mexico: Baja California, San Antonio de las Minas (near Ensenada), on leaves of *Quercus agrifolia*, leg. G. Moreno, 14.III.1990, AH 12634. Baja California, Rancho las Jacarandas (Cañón de las Ánimas), on leaves of *Sambucus mexicana*, leg. M. Lizárraga, G. Moreno & C. Illana, 7.II.1993, AH 15982, 15983, 21004 and 21006.

**Latin diagnosis.** *A Didymium rubeopus capillitio ex filis cylindraceis hyalinis vel albidis compositur differt.*

This variety differs from *Didymium rubeopus* var. *rubeopus* only by the filaments of the capillitium, which are from hyaline to whitish. The remaining macro- and micro-characters and the foliicolous habitat are similar.

Currently, this new variety appears to be more abundant than *Didymium rubeopus*.

## DISCUSSION

Macroscopically, *Didymium rubeopus*, is similar to *D. laxifilum*, but the microscopical characters are distinct. *D. laxifilum* has a brown-violaceous to dark brown capillitium, hyaline at the ends, of thick and rigid filaments (5-8  $\mu\text{m}$  diam.), branched and anastomosed, forming a well defined three-dimensional net and the sporal size are 10-13  $\mu\text{m}$  in diam. (not 9-11  $\mu\text{m}$  in diam. ■ described in latin diagnosis). On the contrary, *D. rubeopus* var. *rubeopus* has a brown to dark brown capillitium formed of more fine filaments (2-4  $\mu\text{m}$  diam.), more or less parallel, little branched, flexuous and with little globose expansion, without forming ■ characteristic net and spores 9-11  $\mu\text{m}$  in diam.

Under SEM, the capillitium of *D. laxifilum* has a smooth surface whereas that of *D. rubeopus* has a rugose surface. The spore ornamentation in *D. laxifilum* is formed by warts which may sometimes merge to form short crests, and totally cover the spore surface in a uniform fashion. In *Didymium rubeopus* the spore ornamentation is formed, according to the terminology of Rammeloo (1974), by 0.5  $\mu\text{m}$  tall pila, sometimes united, which are homogeneously distributed throughout the surface.

*Didymium rubeopus* var. *albocapillitium* differs from the var. *rubeopus* only by its hyaline to whitish capillitium. This variation in the colour of the capillitium has been found to be constant in the Spanish, French and Mexican material. The rest of the macro- and microscopical characters (sporocarp, colour of the stipe, morphology of the capillitium and spore ornamentation) are similar.

*Didymium ovoideum* Nann.-Bremek. also has sporocarps with a reddish stipe but its spores are smaller (6)7-8(9.5)  $\mu\text{m}$  diam (Nannenga-Bremekamp, 1991). A study of the spore ornamentation of this species was made by Gaither & Collins (1984).

*Didymium megalosporum* Berk. & Curt. is a species which has been interpreted in various ways, and which can be confused with the *D. rubeopus* but, after having studied and compared our collections with other exsiccates deposited in American and European herbaria, including the type, we reached the same conclusion as Ing, who also examined the type, deposited in Kew and declared "that it is undoubtedly the same as *D. eximium*" (Martin & Alexopoulos, 1969). *Didymium megalosporum* is a follicolous species which is different from *D. rubeopus* by its flat columella, typically yellowish, with the straw-yellow stipe (Illana & al., 1997).

*Didymium laxifilum* is a species only known from France, England, Spain and the USA (Neubert & al., 1995). It has been collected in Spain on sclerophyllic mediterranean vegetation.

*Didymium rubeopus* and *D. rubeopus* var. *albocapillitium* are also two abundant follicolous taxa on sclerophyllic plants, which may have been confused with the species previously mentioned.

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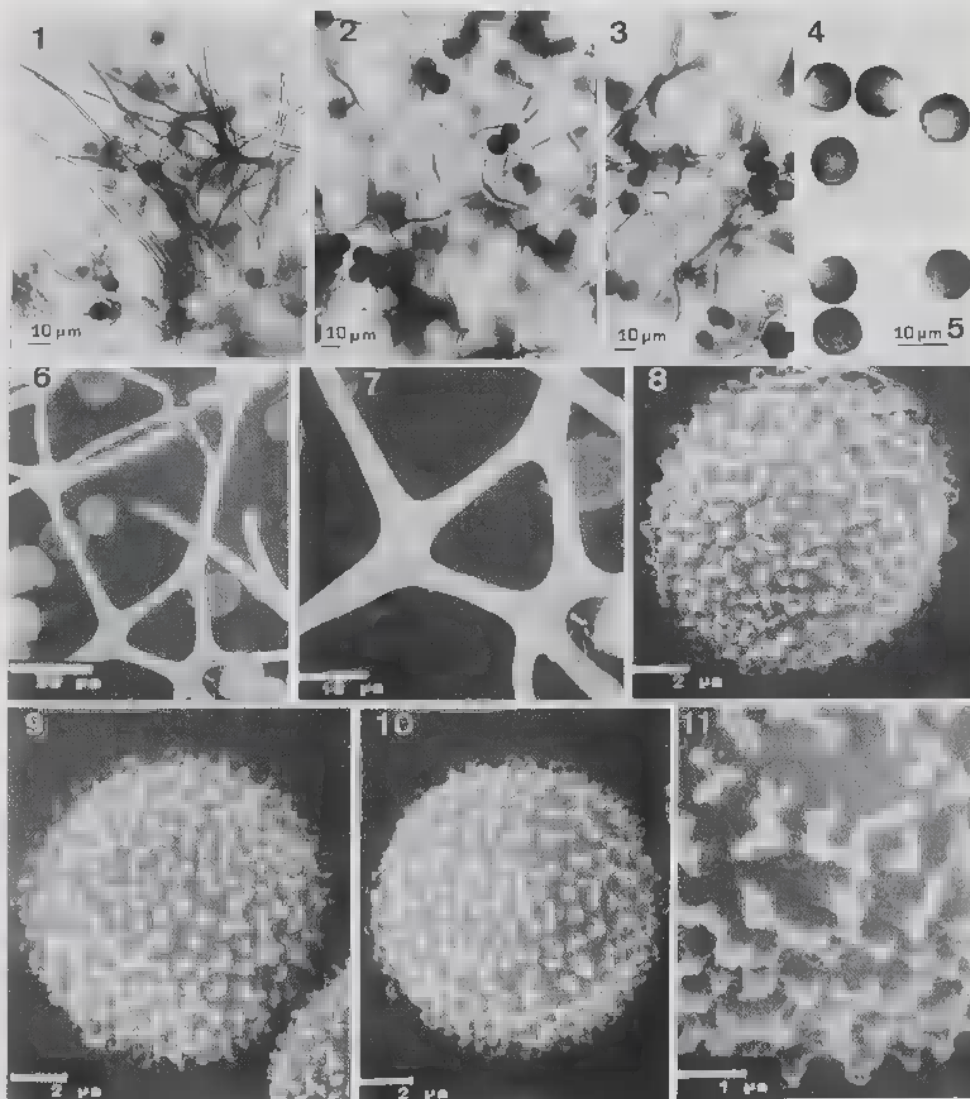
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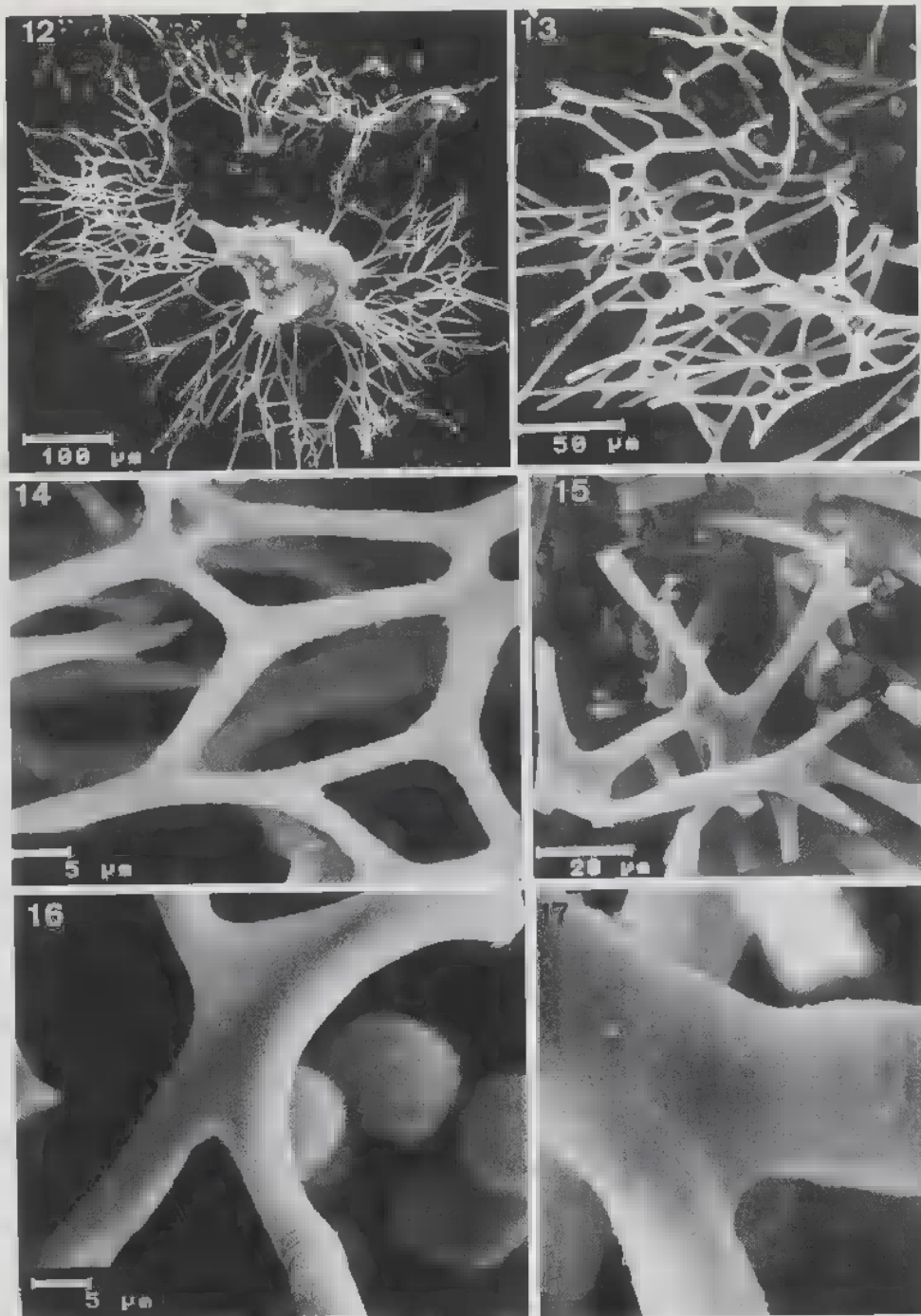
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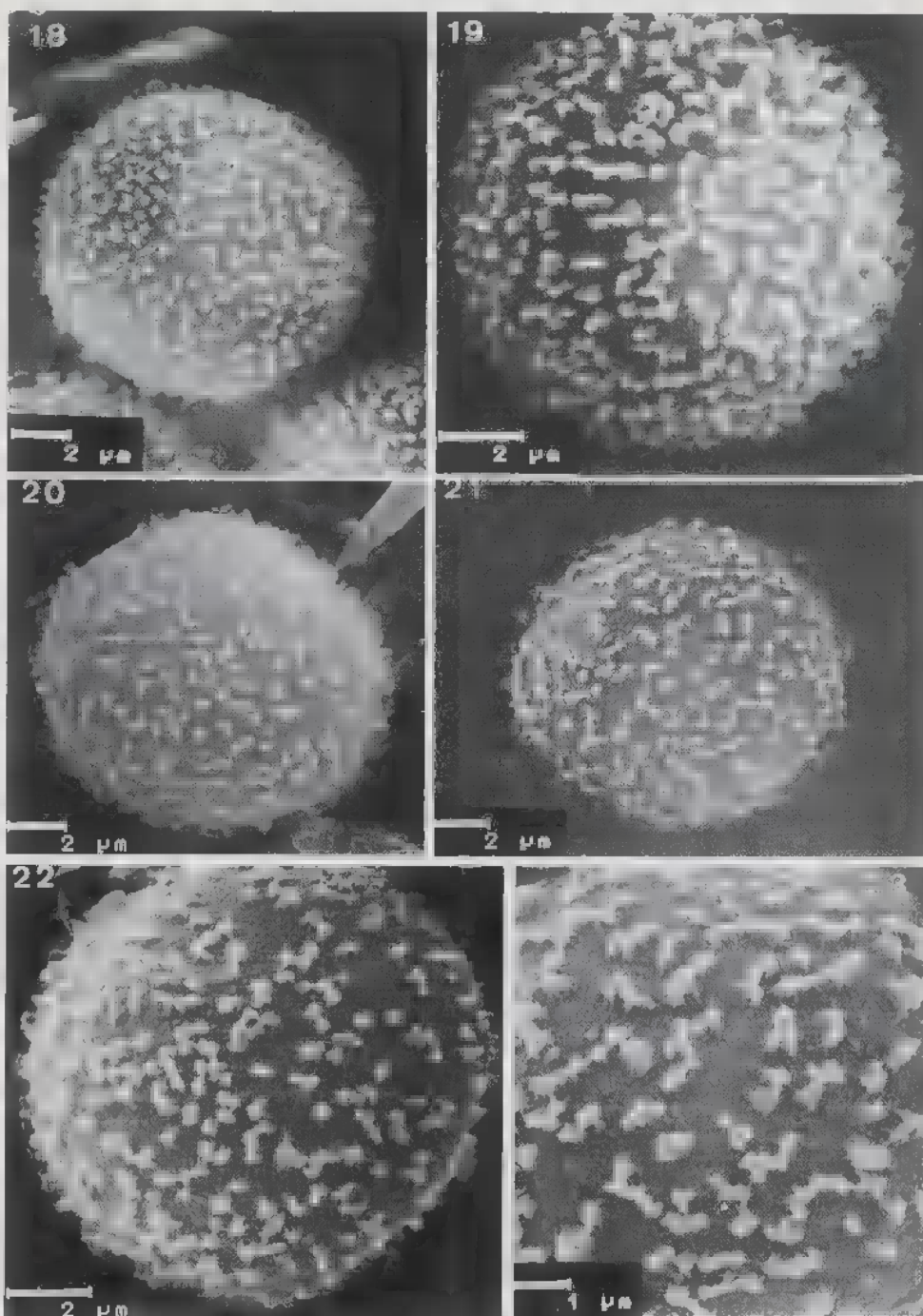


Figs. 1-11. — *Didymium laxifilum* (J. Ross 3501, lectotype). 1-3: detail of the capillitium (LM). 4-5: spores (LM). 6-7: capillitium (SEM). 8-10: spores (SEM). 11: detail of the spore ornamentation (SEM).

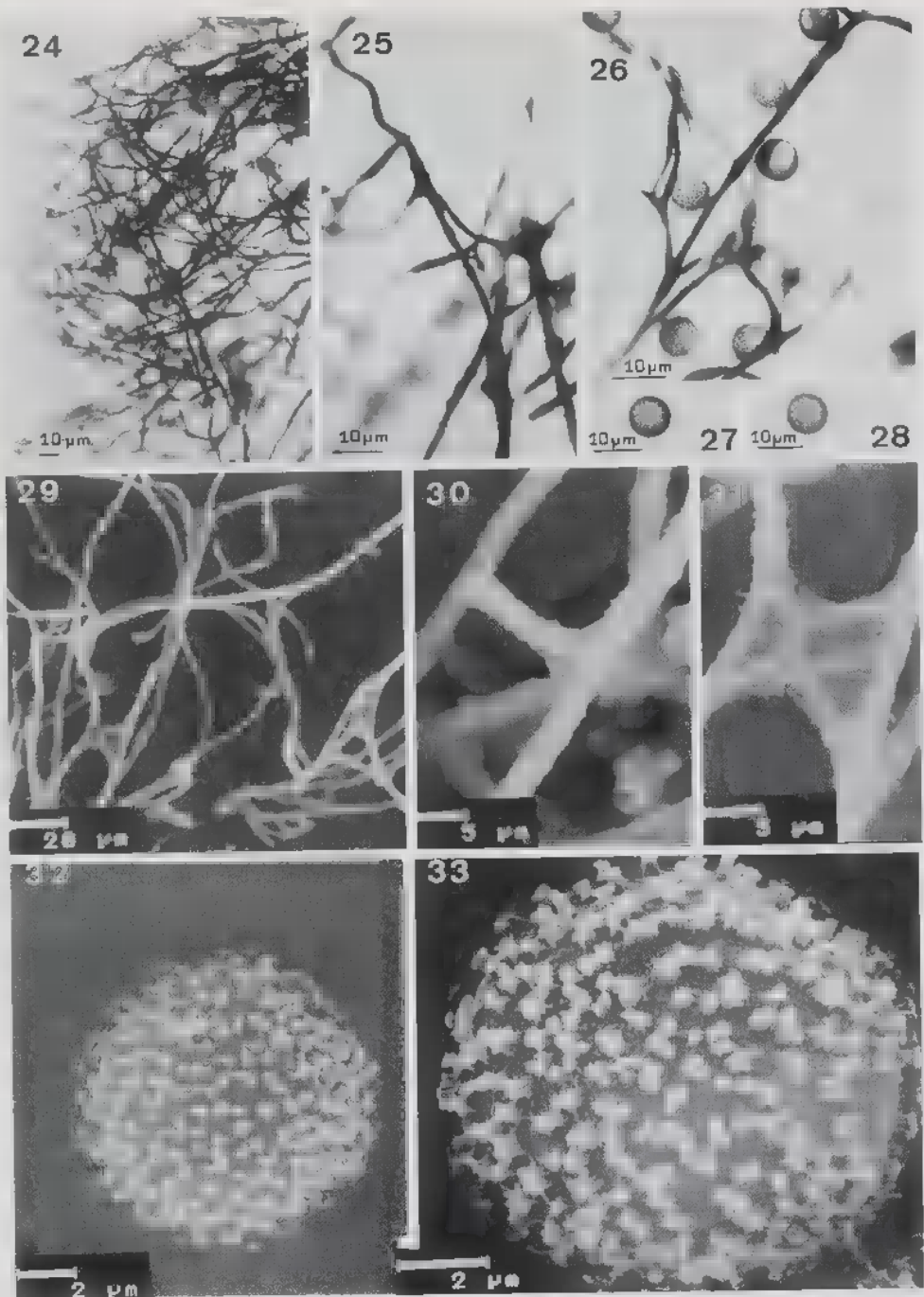


Figs. 12-17. — *Didymium laxiflum*. 12-13: detail del capillitium (J. Ross 3501, lectotype, SEM). 14-15: detail of the capillitium (AH 13383, SEM). 16-17: detail of the capillitium (AH 16429, SEM).

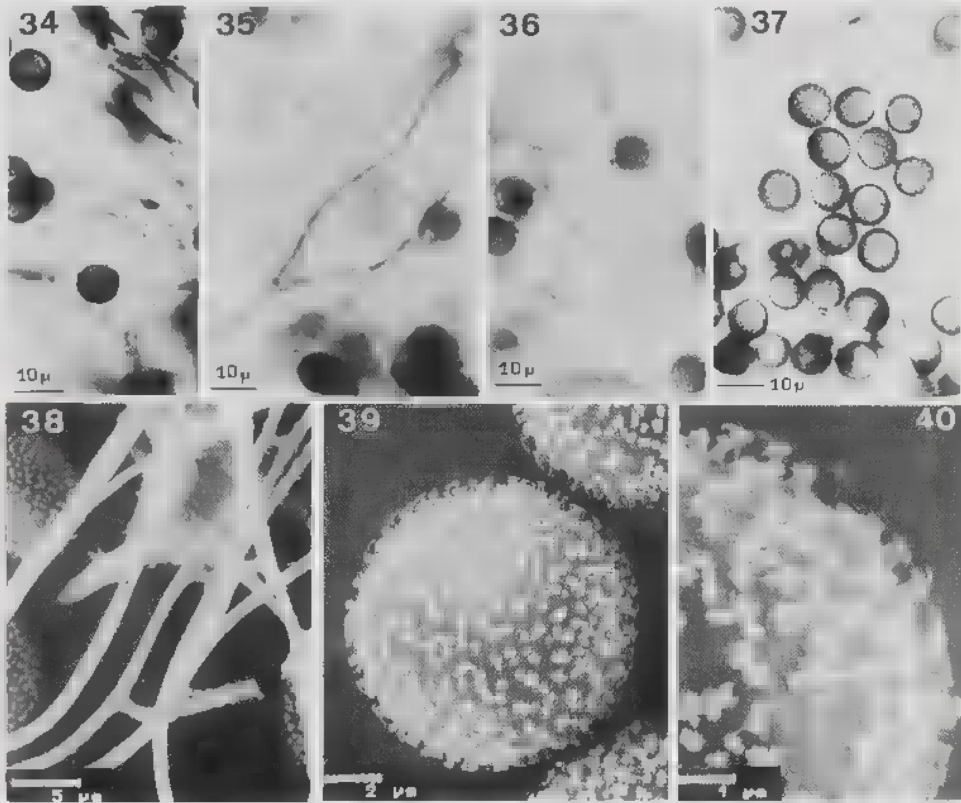




Figs. 18-23 — *Didymium laxifilum*. 18-19: spores (Loughton, the Warren 16702, SEM). 20 — 21: spores (AH 13383, SEM). 22-23: spore and detail of the spore ornamentation (AH 16429, SEM).



Figs. 24-33. — *Didymium rubeopus* var. *rubeopus* (AH 16458, type). 24-26: detail of the capillitium (LM). 27-28: spores (LM). 29-31: detail of the capillitium (SEM). 32-33: spores (SEM).



Figs. 34-40. — *Didymium rubeopus* var. *albocapillitium* (AH 16379, type). 34-36: detail of the capillitium (LM), 37: spores (LM). 38: capillitium (SEM). 39: spore (SEM). 40: detail of the spore ornamentation (SEM).